Friday, March 24, Session IV

L16

QUALITY OF NUCLEIC ACID STRUCTURES IN THE PDB PUBLIC ARCHIVE

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When we compare the publicly accessible structures containing nucleic acids and proteins, we see that the NA-containing structures (i) represent about 8% of the archive, (ii) they have a lower quality, and (iii) that the situation is not improving with time. The quality problems of NA-containing structures can be divided into three groups: (i) inconsistently set and applied target values of the valence geometry, bond distances and bond angles, for nucleotides; (ii) poorly refined backbone geometries; (iii) incompletely and often incorrectly assigned base pairing topologies. In the talk, I will briefly discuss reasons I believe contribute to the lower overall quality of NA-containing versus protein structures and some possible ways to future improvements.

This research was funded by Czech Academy of Sciences, grant RVO 86652036.

L17

WHAT GOVERNS PROTEIN:PROTEIN INTERACTIONS OF C-TYPE LECTIN-LIKE DOMAINS?

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Protein domains with the C-type lectin-like (CTL) fold are widely utilized in important protein:protein interactions. The molecular and cellular context of these interactions covers various systems from snake venoms to human immune system components. The protein:protein interaction affinity for the individual cases differs within six orders of magnitude (!) and all the possible types of protein:protein interactions (hydrogen bonds, shape complementarity etc.) are being used.

For analysis of the CTL-based protein:protein interactions, we used a specific method: mapping of interactions onto one CTL representative (human CD69). This enabled comparison of individual cases of CTL surface utilization and definition of typical classes [1]. Several types of interactions of CTL domains can be distinguished, none of them requiring strict surface sequence conservation. The CTL interaction classification brings a better understanding of this frequently used domain type and draws the basic framework for potential development of protein binders.

Our recent structure of the complex of the human receptor:ligand interaction pair NKR-P1:LLT1 forms an excellent example. The human natural killer cell surface receptor NKR-P1 interacts with its cognate ligand LLT1 in an interesting manner, relying on two CTL:CTL interaction modes (Fig. 1) [2]. This interaction is one of the key components in recognition of tumour or virus-infected cells by immune system. The pair represents one of the CTL:CTL interactions with low affinity and several typical features of the "Canonical CTL:protein interaction" [1]. Comparison of the hNKR-P1:LLT1 interaction with other

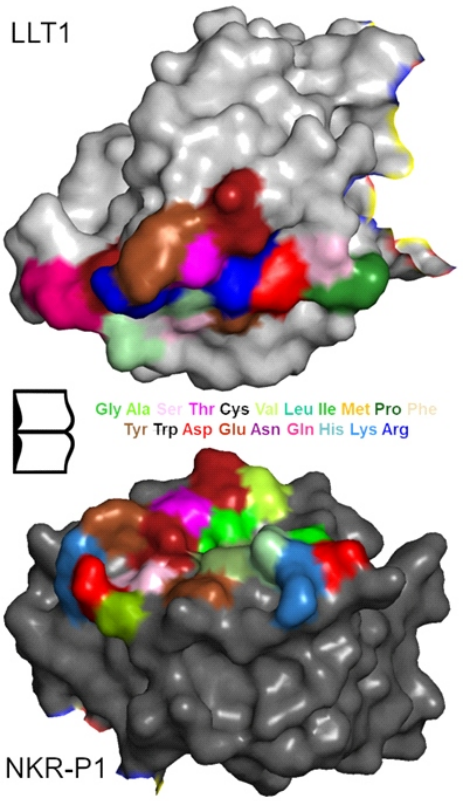

Figure 1. Primary interaction interface of human NKR-P1 and its ligand LLT1. An "open book" view with the interacting residues coloured according to amino acid type.

similar mammalian interaction pairs conforms to our structural classification of the CTL:CTL interactions.

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This work was supported by the Czech Science Foundation (18-10687S), by the ERDF fund (CZ.02.1.01/0.0/0.0/16_013/0001776 and CZ.02.1.01/0.0/0.0/15_003/0000447), and by the Czech Academy of Sciences (86652036).

L18

STRUCTURAL CHARACTERIZATION OF FOXO4-DBD:p53-TAD INTERACTION

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The transcription factor p53 protects cells against tumorogenesis when exposed to various cellular stresses. Under these conditions, p53 interacts with the transcription factor FOXO4 to induce cellular senescence, a condition contributing significantly to the aging process, by upregulating the transcription of the senescence-associated protein p21 [1]. However, the structural details of FOXO4:p53 interaction still remain unclear. Inhibition of this interaction has been shown to help restore homeostasis of senescent tissues and counteract signs of aging, making it a target for the design of senolytic compounds [2].

In previous published work, we characterized the FOXO4:p53 binding interface and showed that the DNA-binding domain (DBD) of FOXO4 and the transactivation domain (TAD) of p53 are essential for the stability of this protein-protein complex [3]. In this work, we characterize the interaction between p53TAD and FOXO4-DBD. We first determined the affinity of the complex using fluorescence anisotropy measurements. Subsequently, we performed a set of paramagnetic relaxation rate enhancement experiments (PRE) combined with ^{15}N relaxation experiments. The PRE results allowed us to derive a set of distance constraints that, together with R_2/R_1 values obtained from ^{15}N relaxation experiments and significant CSP values from the ^{1}H-^{15}N HSQC titrations, were used to build a HADDOCK model of the FOXO4-DBD:p53-TAD complex.

Our structural insights may help to understand the interrelated functions of FOXO4 and p53 in cellular homeostasis, longevity, and stress response, as well as to further help the develepment of senolytic compounds.

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This work was supported by Czech Science Foundation Grant No. 21-02080S, the Grant agency of the Charles University (project number: 296621) and the Czech Academy of Sciences (Research Project RVO: 67985823 of the Institute of Physiology).

L19

INSIGHT INTO INHERITED ANEMIA CDA-I: DISEASE-ASSOCIATED MUTATIONS DISRUPT CODANIN1-CDIN1 COMPLEX

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Congenital dyserythropoietic anemias (CDAs) cause ineffective erythropoiesis and morphological anomalies in erythrocytes and erythroblasts [1]. One of them, CDA type I (CDA-I), is rare hereditary anemia described by congenital abnormalities like interchromatin bridges and Swiss-cheese-like heterochromatin [2].

CDA-I is associated with mutations in two different loci, *CDAN1* and *CDIN1*. *CDAN1*, encoding Codanin1, is involved in nucleosome assembly and disassembly [3]. CDIN1 is a recently discovered protein predicted to be a divalent metal ion-dependent restriction endonuclease [4]. Despite their undeniable importance for CDA-I progression, both proteins and their mutual interaction are poorly described.

Here, we present a pioneer study of the essential interaction between CDIN1 and Codanin1. Firstly, we characterized the biophysical properties of both proteins. We investigated their homodimerization and heterodimerization and their structural features. Additionally, we quantified CDIN1-Codanin1 binding affinity in the low nanomolar range. Finally, we defined CDIN1-Codanin1 interaction regions and showed that CDA-I-related mutations residing in identified interaction regions disturb the CDIN1 Codanin1 complex.

The results of this project are an essential step toward unraveling the CDA-I activation and progression process that will be employed in the future design of biological therapy.

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TB is supported by Brno Ph.D. Talent Scholarship - funded by Brno City Municipality. The research has been carried out with the support of the Czech Science Foundation (GA23-05241S). We acknowledge CEITEC Proteomics Core Facility of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127). We acknowledge CF Biomolecular Interactions and Crystallization of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127). We acknowledge CMS-Biocev ("Biophysical techniques, Crystallization, Diffraction, Structural mass spectrometry") of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and CZ.02.1.01/0.0/0.0/18_046/0015974.

L20

SUPR-DSF - DIFFERENTIAL SCANNING FLUORIMETRY FOR HIGH-THROUGHPUT PROTEIN STABILITY SCREENING

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The presentation will cover an introduction into the new SUPR-DSF system from Protein Stable and a brief overview of Chirascan CD spectrometers from Applied Photophysics. Both systems can aid in stability screening of proteins and in the biophysical characterisation of proteins.

Differential Scanning Fluorimetry (DSF) is a valuable and widely-used technique that monitors protein unfolding with increasing temperature by detecting changes in fluorescence. However, the conventional workflow for DSF uses extrinsic dyes that may influence the thermal stability of the protein under investigation. This can affect the quality of your data by generating false positives or negatives during screening. The SUPR-DSF system from Protein Stable measures intrinsic fluorescence of proteins to detect structural changes and avoids the use of additional dyes. The SUPR-DSF provides fluorescence measurement data directly from 384-well plates and with no proprietary consumables. SUPR-DSF reduces operator time and minimizes the risk of errors in multi-step sample preparation while also bringing down the cost of consumables and sample consumption without any compromise to data quality.

Our Chirascan CD spectrometers contribute to a deeper understanding of biomolecular characteristics, mechanisms and interactions. Our system can be used not only to gain insight and detect changes in secondary and tertiary structure but to study folding and unfolding mechanisms during altered physiological parameters. The Chirascan systems can also be fitted with additional accessories to expand its capabilities further.